GENETIC ANALYSIS OF THE 5S RNA GENES IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

The 5S RNA genes of *Drosophila melanogaster* in either an isogenic wild-type or a multiply inverted (*SM1*) chromosome *2* increase their multiplicity when opposite a deficiency for the 5S gene site. This is analogous to the compensation phenomenon previously described for the 18S and 28S ribosomal RNA genes of the *X* chromosome nucleolus organizer region. Molecular hybridization of 5S RNA to DNA containing various doses of the 56F1–9 region of chromosome *2* demonstrates that most, if not all, of the 5S genes reside in or near this region. Also, a deficiency missing approximately one-half of the wild-type number of 5S genes was isolated and genetically localized. This mutant has a phenotype like that of bobbed, a mutant known to be partially deficient in 18S and 28S ribosomal RNA genes. Finally, we report the existence of a chromosomal rearrangement which splits the second chromosome into two segments, each containing 5S DNA.

IN *Drosophila melanogaster*, there are approximately 200 copies of the 5S RNA gene per haploid genome (TARTOF and PERRY 1970), each gene coding for a 5S RNA molecule found in the ribosome. Utilizing *in situ* hybridization techniques, WIMBER and STEFFENSON (1970) localized the 5S genes to the 56F1–9 region of chromosome *2*. Similarly, in *Homo sapiens* these genes are clustered at a single site on chromosome *1* (JOHNSON, HENDERSON and ATWOOD 1974). In contrast, the 5S genes of *Xenopus laevis* are localized on the telomeric regions of many, if not all, of the 18 chromosomes (PARDUE, BROWN and BIRNSTIEL 1973). The localization of the 5S genes to a single euchromatic site in *D. melanogaster* enables the investigation of regulatory processes governing this redundant locus. Utilizing available deficiencies for the 56F1–9 region (LINDSLEY and SANDLER *et al.* 1972) readily allows the study of gene compensation (TARTOF 1971), magnification-reduction (RITOSSA 1968; TARTOF 1974) and the isolation of various mutations that affect 5S RNA synthesis. In addition, the euchromatic nature of the locus permits classical recombination analysis and cytological examination of 5S mutants.

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This report presents evidence that the euchromatic 5S RNA genes of *D. melanogaster* can increase their number when the homologous chromosome is completely deficient for 5S DNA. This is analogous to the compensation phenomenon initially described for rDNA of the nucleolus organizer region (NO) (TARTOF 1971). However, we show that it is possible to avoid the compensation effect and obtain accurate quantitation of 5S gene numbers by the use of appropriate genotypes. A duplication and four deficiencies for the 56F1–9 region were constructed and molecular hybridization results confirm the *in situ* hybridization data that the majority, if not all, of the 5S genes are localized within or near this region. In addition, a partial deletion of the 5S genes was isolated and mapped by means of both recombination and deletion analysis.

MATERIALS AND METHODS

a) Drosophila melanogaster *stocks*

Cultures of *D. melanogaster* were reared at 25° ± 0.5° in half-pint bottles using a cornmeal, agar, and molasses medium (TARTOF 1973).

The construction of segmental aneuploids for the 56F1–9 region was accomplished as follows (LINDSLEY and SANDLER *et al.* 1972). *C(1)RM,y; T(Y;2)$_x$/In(2LR)SM1, al^2 Cy cn^2 sp^2* females were crossed to $Y^S X{\cdot}Y^L$, *In(1)EN, y; T(Y;2)y/Sco* males, where *x* and *y* represent translocations with different autosomal breakpoints but both have a breakpoint in the Y^S arm of the *Y* chromosome. The translocated *Y* chromosome is marked with B^S on Y^L and *y*+ on Y^S. The *C(1)RM*, $Y^S{\cdot}X{\cdot}Y^L$ and *In(2LR)SM1* chromosomes are symbolized as $\overline{XX}$, and $\overline{XY}$ and *SM1*, respectively. The translocations used here were G100, L139, L62 and L141 (LINDSLEY and SANDLER *et al.* 1972). Adjacent-1 disjunction male progeny $\overline{XY}$; *T(Y;2)$_{x-y}$/SM1* receive the B^SY chromosome fragment from one parent and the *y*+ fragment from the other. Thus these males have either a duplication or deficiency for the 56F1–9 region, depending upon which translocations were present in the mother and father. Stable stocks were made from these aneuploid males as $\overline{XX}/\overline{XY}/T(Y;2)_{x-y}/SM1$ and the stocks were selected for appropriate markers at every generation. The deficiency G100-L141 stock was synthesized by DR. DAN LINDSLEY; duplication G100–L141 and deficiencies L139–L141, L62–L141 and L139–L62 stocks were synthesized in this laboratory (Figure 2). Deficiency G100–L141 and duplication G100–L141 can be genetically distinguished from each other in that *min/Df$_{G100-L141}$* flies have a *min* phenotype but *min/Dp$_{G100-L141}$* are wild type.

Five different genotypes were used to prepare DNA containing various doses of the 5S genes (Figure 1). Isogenic Oregon-R males and females have two doses of the 5S genes, one on each second chromosome. Flies having a single dose were constructed by mating isogenic Oregon-R males to $\overline{XX}$; *T(Y;2) Df$_{G100-L141}$/SM1* females (*Df$_{G100-L141}$* designates a deficiency for the 56F1–9 region). Male progeny *X*; *T(Y;2) Df$_{G100-L141}$*/+ have one dose of the 5S genes. The daughters of this cross are $\overline{XX}/Y$; *SM1*/+ and have two doses of 56F1–9. Oregon-R males were mated to $\overline{XX}$; *T(Y;2) Dp$_{G100-L141}$/SM1* females (*Dp$_{G100-L141}$*, a duplication for 56F1–9) to produce *X*; *T(Y;2) Dp$_{G100-L141}$*/+ males that have three doses of the 5S genes. Females and males from the stock $\overline{XX}/\overline{XY}$/*T(Y;2) Df$_{G100-L141}$/SM1* were also used as a single dose of 56F1–9. A complete description of Drosophila genetic terminology is given in LINDSLEY and GRELL (1968).

b) *Genetic mapping*

The *min* allele was mapped genetically with the dominant markers *Pfd* (Pufdi, 70.8) and *Px* (Plexate, 107.2) on chromosome 2. *Pfd Px/min* virgin females were mated to $\overline{XY}$; *T(Y;2) Df$_{L139-L141}$/SM1* males and all the $B^S y^+$ (*Df$_{L139-L141}$*) chromosome-bearing male progeny were scored.

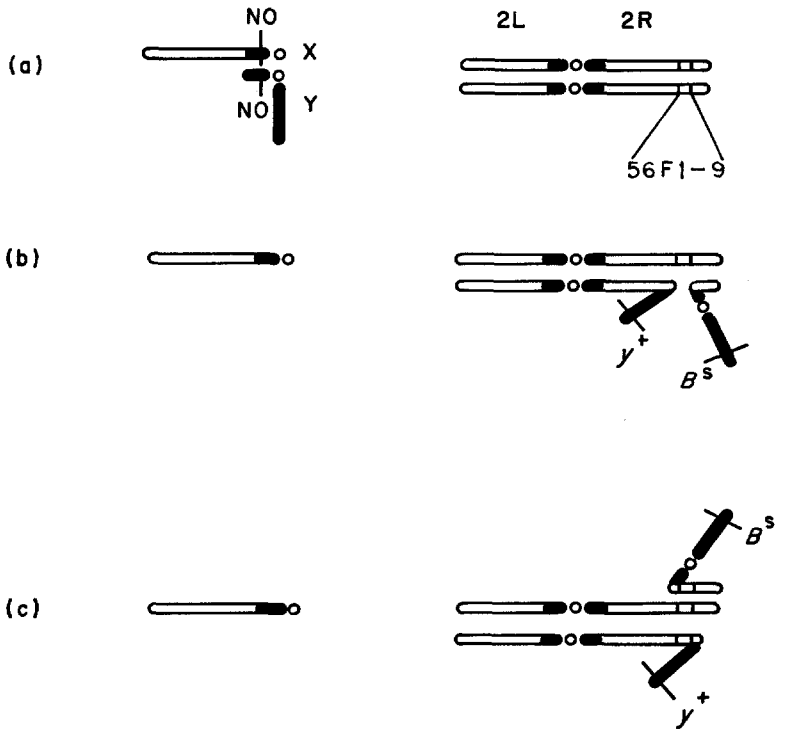

FIGURE 1.—Structure of sex and second chromosomes in males having various doses of the 56F1–9 region. A—Oregon-R males, with an *X* and *Y* chromosome each containing a NO-region. Females have two *X* chromosomes. Each second chromosome of both sexes has one dose of 56F1–9 positioned on the right arm (*2R*). The solid regions designate centric heterochromatin; B—$X/T(Y;2)Df_{G100-L141}/+$ males having one dose of 56F1–9. The translocated *Y* chromosome is marked with B^S on the long arm (Y^L) and *y*+ on the short arm (Y^S); *C*—$X/T(Y;2)$-$Dp_{G100-L141}/+$ males with three doses of the 56F1–9 region.

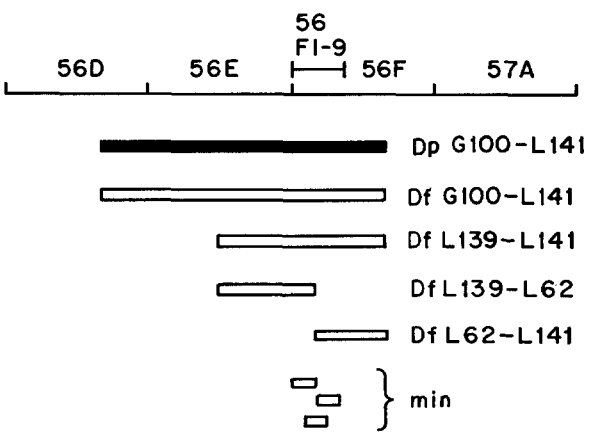

FIGURE 2.—The segmental aneuploids for the 56D–56F region of chromosome *2*. The solid bar represents the duplication; the open bars indicate the deficiencies. The *min* allele is designated as one of three possible deletions.

c) *DNA isolation*

DNA was isolated from flies by the MUP method of BRITTEN, PAVICH and SMITH (1969). Approximately 20–30 g of frozen adult flies (—70°) of the appropriate genotype were homogenized at 4° in 150 ml of a lytic solution (0.1 M EDTA, pH 8.0, 2% sodium dodecyl sulfate, 0.5 M sodium perchlorate and 0.15 M NaCl) and 150 ml of water-saturated phenol (pH 8.0) containing 0.1% (w/v) 8 -hydroxyquinoline. The homogenate was shaken for 20 minutes and centrifuged at 1500 g for 10 minutes. The aqueous phase was reextracted with an equal volume of chloroform-isoamyl alcohol (24:1). The DNA was precipitated with 1 volume of cold 95% ethanol, centrifuged and redissolved in 15 ml of MUP (8 urea and 0.24 M neutral prosphate buffer, PB). The DNA was absorbed onto a hydroxyapatite column (4 g of HAP, Bio-Rad HTP, in 0.24 M PB) and the column washed first with MUP, then with 0.014 M PB. The DNA was eluted from the column with 0.40 M PB, dialyzed against 0.1 × SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and stored at —70°. The 18S and 28S rRNA-DNA hybridization values for DNA of a specific genotype prepared in this manner were identical to that of DNA prepared by the phenol-enzyme digestion method previously used in this laboratory (TARTOF 1973).

d) *RNA extractions*

^{3}H-labeled and unlabeled 18S and 28S RNA were prepared by the method of TARTOF and PERRY (1970). The ^{3}H-labeled 5S RNA was isolated from *D. melanogaster* cell culture line No. 2 (SCHNEIDER 1972). Cells were grown in spinner culture containing SCHNEIDER's medium supplemented with 15% fetal calf serum and 5.0 mg/ml bactopeptone. The cells were incubated with 10 μCi/ml ^{3}H-uridine (27.8 Ci/m-mole, New England Nuclear Corp.) for 24 hours; then an additional 10μCi/ml was added. After 72 hours the cells were washed twice in insect Ringers solution (128 mM NaCl, 4.67 mM KCl and 2 mM $CaCl_2$) with 5 μg/ml polyvinylsulfate (Sigma) and lysed in 10 mM sodium acetate (pH 6.0), 12 mM $MgCl_2$, 150 mM KCl, 0.5% Triton-X100 and 10 μg/ml polyvinylsulfate. The lysate was centrifuged and to the supernatant sodium dodecylsulfate (SDS) was added to a final concentration of 1% and then shaken for 20 minutes with an equal volume of phenol-chloroform (1:1 v/v containing 0.1% 8-hydroxyquinoline) saturated with ANE buffer (0.01 M acetate, pH 6.0, 0.1 M NaCl and 0.001 M EDTA). The aqueous phase was recovered by centrifugation and reextracted with phenol-chloroform two more times. RNA was precipitated with two volumes of cold 100% ethanol, centrifuged and redissolved in ANE buffer at a concentration of 1.0–1.5 mg/ml. The 18S and 28S RNA was precipitated by adding solid NaCl to a concentration of 2.0 M and allowing the solution to stand overnight at 2°. After centrifugation, 4S and 5S RNA in the supernatant was precipitated with 100% ethanol. The 4S and 5S RNA was collected by centrifugation and dissolved in E buffer (0.04 M Tris, pH 7.2, 0.02 M sodium acetate, 0.001 M EDTA) containing 0.2% SDS and 20% sucrose.

The 4S and 5S RNA was preparatively separated in 10.0% acrylamide—0.25% bis-acrylamide gels (9.2 cm long, 0.9 cm diameter) polymerized in E buffer (LOENING 1967). Electrophoresis was continued for 2 and ¾ hours at 10 mA/gel in E buffer containing 0.2% SDS. The 4S and 5S RNA bands, readily observed under ultraviolet light (Mineralight, UVSL-25, Ultra-Violet Products, Inc., short-wave setting), were cut out with a razor blade. The separated 4S and 5S RNA's were recovered from the gel slices by electrophoresis and dialyzed against 2 × SSC. The RNA solution was then centrifuged at 14,000 g for 30 minutes at 2° and passed through a nitrocellulose filter (Schleicher and Schuell, B6) in order to remove a contaminating acrylamide-bisacrylamide precipitate. Approximately 120 μg of 5S RNA (1.17×10^5 cpm/μg) were isolated in this manner from 1.5×10^9 cells.

e) *DNA-RNA hybridization*

Preparation of filter-bound DNA and 18S + 28S ^{3}H-RNA hybridization was done by the method described by PROCUNIER and WILLIAMSON (1974). The 5S ^{3}H-RNA hybridization reactions were carried out for 4 hours at 60° by placing three circular filters (7 mm in diameter) in a vial containing 0.2 ml of 2 × SSC with 0.46, 0.69, 0.92 or 1.38 μg of ^{3}H-labeled 5S RNA and 100 times these amounts of unlabeled 18S and 28S RNA. Saturation was achieved at these inputs

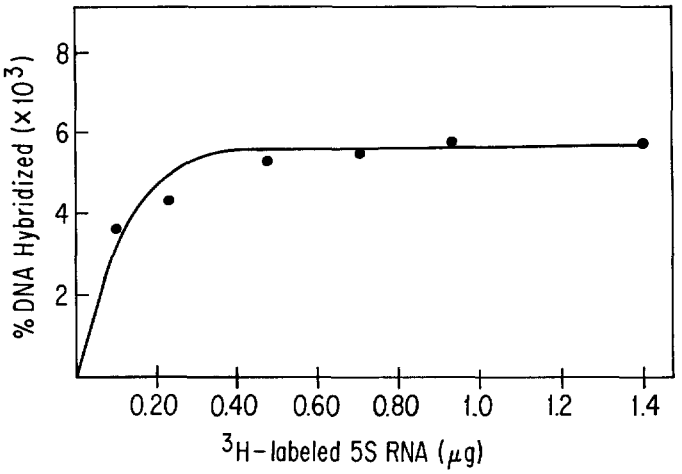

FIGURE 3.—Saturation of Drosophila DNA with ^{3}H-labeled 5S RNA. Filters containing 50 μg of DNA from Oregon-R males and females incubated with ^{3}H-labeled 5S RNA (1.17×10^5 cpm/μg) and a 100-fold excess of unlabeled 18S and 28S RNA. Hybridization was carried out in 0.20 ml of 2 × SSC at 60° for 4 hours.

of 5S RNA (Figure 3). An excess of unlabeled 18S and 28S RNA was added to dilute out any degraded 18S and 28S ^{3}H-RNA present in the 5S RNA preparation. At the end of the reaction time the filters were washed with 2 × SSC at 60° and treated for 1 hour at 37° with 20 μg/ml RNase in 2 × SSC (Sigma, heated for 10 minutes at 95°). Following another washing, the filters were dried out at 60° and counted in 5 ml of Liquifluor (New England Nuclear Corp., diluted 1:25 with toluene). The number of ^{3}H-labeled 5S RNA counts for 50μg of wild-type DNA was approximately 330 cpm and background values (identically treated filters but containing no DNA) were less than 10 cpm. The hybridization values for each particular genotype were averaged and the standard error (SE) calculated. The hybridization values for genotypes with an extra *Y* chromosome were multiplied by 1.1 to correct for the 10% extra DNA and render the hybridization values from these genotypes directly comparable to the wild-type DNA content. The number of 18S + 28S rRNA and 5S RNA genes for each genotype was calculated using the molecular weights of the diploid genome of *D. melanogaster*, 18S + 28S RNA and 5S RNA as 2.4×10^{11}, 2.1×10^6 and 4.0×10^4, respectively (TARTOF and PERRY 1970).

RESULTS AND DISCUSSION

In order to eliminate genetic variability when measuring the number of 5S genes in chromosome *2*, an isogenic wild-type strain (inbred for 30 generations) was used. Also, different genotypes which had a wild-type chromosome *2* received this chromosome from the inbred strain. Wild-type isogenic Oregon-R flies which have two doses of the 56F1–9 region have an average of 165 5S genes per chromosome *2* (Table 1). Males and females have an equal number of 5S genes (TARTOF and PERRY 1970). The 5S RNA-DNA hybridization values for DNA extracted from three separate populations of isogenic Oregon-R flies were 0.0052%, 0.0060% and 0.0054%, with a mean value of 0.0055%. These values are in close agreement and ensure the reproducibility of measuring 5S gene numbers. This value is consistent with the average value of 0.007% previously reported (TARTOF and PERRY 1970).

TABLE 1

The number of 5S RNA genes for various second chromosomes

Genotype of chromosome *2*	No. 56F1–9 regions	DNA hybridized (% × 10^5 ± SE)	Total no. 5S genes	No. 5S genes per 56F1–9 region	Compensatory net increase of 5S genes	No. determinations
+/+ (Oregon-R isogenic)	2	520 ± 28	312	156	—	4
		600 ± 36	360	180	—	4
		540 ± 25	324	162	—	4
	Average	553 ± 19	331	165	—	12
$+/Df_{G100-L141}$	1	442 ± 16	265	265	100	4
$+/Dp_{G100-L141}$	3	801 ± 31	480	160	—	7
+/SM1	2	542 ± 15	325	162	—	4
$SM1/Df_{G100-L141}$	1	481 ± 41	288	288	126	4

When the same 56F1–9 region is placed opposite a deficiency for this region, as in *X;T(Y;2)* $Df_{G100-L141}$/+ males, the number of 5S genes is 265 (Table 1). Thus, there is an increase in 5S gene multiplicity of approximately 60% when the 56F1–9 region is present as a single dose. This result is also demonstrated for the 56F1–9 region of the *SM1* chromosome, a multiply-inverted chromosome *2*. The *SM1* chromosome in $\overline{XX}/Y;SM1/+$ females has the same number of 5S genes as the wild-type chromosome *2*. When the *SM1* chromosome is opposite a deficiency for 5S genes, its 5S gene multiplicity is increased by approximately 76% seen in $\overline{XY}/\overline{XX}/T(Y;2)$ $Df_{G100-L141}/SM1$ males and females. The 5S genes of both the wild-type chromosome *2* and the *SM1* chromosome compensate when opposite a deficiency. An alternative explanation for the number of 5S genes not being proportional to the number of 56F1–9 regions in this case could be that there is an additional 5S gene site(s) not covered by the deficiency. In order to eliminate this possibility, a duplication for the 56F1–9 region was constructed. Males of the genotype *X;T(Y;2)* $Dp_{G100-L141}/+$ have three doses of the 56F1–9 region and 480 5S genes. Subtracting the number of 5S genes contributed by the wild-type chromosome *2* gives 320 genes for the duplication-bearing translocation chromosomes which have two doses of the 56F1–9 region. The 5S gene multiplicity is proportional to the number of 56F1–9 regions in this case. Therefore, the increased number of 5S RNA genes in the case of $+/Df_{G100-L141}$ must be the result of a compensatory mechanism analogous to that previously reported for the rRNA genes (TARTOF 1971).

It is evident from these observations that when determining the 5S or 18S and 28S gene multiplicity of a particular chromosome, ambiguous results may occur as a consequence of compensation. In order to determine the true multiplicity of a redundant locus, appropriate genetic conditions must be used. When measuring the rDNA content of a particular *bb* allele, this allele is placed opposite a *Y* chromosome, the *Y* chromosome NO region being refractory to compensation (TARTOF 1971; PROCUNIER and WILLIAMSON 1974). In order to measure the 5S multiplicity of a particular chromosome *2*, it may be placed opposite a duplication

TABLE 2

The number of 5S RNA genes for the min *allele*

Genotype of chromosome *2*	No. 56F1–9 regions	DNA hybridized (% × 10^5 ± SE)	Total no. 5S genes	No. 5S genes per *min* allele	No. determinations
min/min	2	295 ± 18	177	88	6
min/$Dp_{G100-L141}$	3	705 ± 47	423	103*	4

* Assuming the duplication-bearing translocation chromosomes contribute 320 genes as measured in +/$Dp_{G100-L141}$ males (Table 1).

for the 56F1–9 region. The number of 5S genes for the duplication-bearing translocation chromosomes opposite either a partial deficiency (*min*) or a wild-type chromosome *2* is consistent with the expectation that the translocation chromosomes contribute the same number of 5S genes in each case (Tables 1 and 2).

A new mutant in *D. melanogaster*, mini (*min*), was isolated and subsequently shown to be a partial deletion for the 5S genes. Since partial deletions of 18S and 28S RNA genes manifest a *bb* phenotype (short, thin bristles, occasional abdominal etching, semi-lethality and a marked increase in developmental time) in the adult fly (RITOSSA, ATWOOD and SPIEGELMAN 1966), males of the genotype *X;T(Y;2)* $Df_{G100-L141}$/+ were screened for a similar phenotype. One dose of the 56F1–9 region has enough 5S genes for a wild-type phenotype. More than 200 second chromosomes from stocks maintained at ICR were examined over the $Df_{G100-L141}$ chromosome, and a male exhibiting phenotypic characteristics identical with those of *bb* mutants was isolated. We refer to this mutation as *min*. The origin of the *min* allele is speculative since the stock from which this mutant came was polymorphic for *min* and min^+ and the history of the particular chromosome *2* harboring the *min* allele is unknown. Flies homozygous for *min* are nearly wild type, demonstrating the additivity of two alleles. The number of 5S genes for the *min* allele measured in *min/min* males and females is 88, a 47% deficiency of the wild-type number (Table 2). This is further substantiated by the fact that males of the genotype *X;T(Y;2)* $Dp_{G100-L141}$/*min* have 103 5S genes for the *min* allele (Table 2). Flies hemizygous for bobbed alleles which have approximately a 40% deficiency of the NO region are phenotypically indistinguishable from *min/*$Df_{G100-L141}$ flies. Moreover, the number of 18S and 28S rRNA genes for *min/min* flies is nearly the same as wild-type isogenic Oregon-R flies and hence, the appearance of the *min* phenotype cannot be due to a partial deletion of rDNA (Table 3).

TABLE 3

The number of 18S and 28S rRNA genes for isogenic wild-type and min *flies*

Genotype	No. NO regions	DNA hybridized (% ± SE)	No. rRNA genes per NO	No. determinations
wild type ♂ ♀	2	0.395 ± .014	225	11
min/min ♂ ♀	2	0.360 ± .022	205	4

Using the second chromosome dominant markers Pufdi (*Pfd*, 70.8) to the left of 56F1–9 and Plexate (*Px*, 107.2) to the right of 56F1–9, the *min* allele mapped genetically at position 90 ± 1.0 and was also found to be allelic to both $Df_{G100-L141}$ and $Df_{L139-L141}$ (Figure 2). Therefore, both genetic and deficiency mapping of the *min* allele place the 5S genes in the vicinity of 56F1–9 of chromosome *2* in conformity with the *in situ* hybridization result. Cytological examination of polytene nuclei of *min/min* and *min/+* flies indicates no gross inversions, translocations or duplications in the 56F1–9 region. Attempts to correlate the *min* 5S DNA deficiency with a specific cytological deletion are being pursued.

The breakpoint of the L62 translocation is most interesting. Males of the genotype *X;T(Y;2)* $Df_{L62-L141}$*/min* and *X;T(Y;2)* $Df_{L139-L62}$*/min* are wild type. Thus, both deficiencies L139–L62 and L62–141 (Figure 2) leave sufficient 5S RNA genes remaining so that, when opposite the *min* chromosome, the fly has a wild-type phenotype. From these results, it is most likely that the L62 breakpoint splits the chromosome into two segments, each containing approximately equal numbers of 5S RNA genes. The complementation of the *min* allele, a 47% deletion of 5S DNA, with both deficiencies indicate that the remaining 5S genes of each deficiency-translocation are functional. This is analogous to the observation that a single NO region can be split to produce two functional nucleolus organizers (McCLINTOCK 1934; SHALET 1969). The 56F1–9 region on BRIDGE's map is comprised of nine bands, four prominent doublets (56F1–2, 56F3–4, 56F6–7 and 56F8–9) and a light band (56F5). Recent *in situ* hybridization evidence suggests there may be two discernible 5S sites in this region (SZABO 1974). The complementation data presented here do not discriminate whether the *L62* breakpoint splits a single cluster of 5S RNA genes or simply separates two already existing and discrete clusters of 5S DNA.

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